Supplemental Material 1

Multicenter prospective randomized clinical trial of neoadjuvant chemoradiation therapy combined with immunotherapy for MSS ultra-low rectal cancer (CHOICE II)

INFORMED CONSENT FORM

Version NO.1.3(20230210)

Research Institute: Shanghai Changhai Hospital

Principal Investigator: Prof. Wei Zhang
1. Purpose of the study

Background

Ultra-low rectal cancer refers to tumors whose lower edge is within 2 cm of the dentate line. Surgical treatment for these patients requires removal of the rectum or anus, which has a high disability rate and disease burden. Neoadjuvant radiotherapy can significantly reduce the size of the tumor, thereby increasing the patients’ chances of preserving the anus. Approximately 20% of patients will experience complete tumor disappearance (complete clinical response) after neoadjuvant radiotherapy. The Watch & Wait nonsurgical strategy for these patients can effectively increase the rate of anus preservation and rectum preservation, and thus improve the quality of patients’ life. The proportion of patients who achieve complete clinical response under current neoadjuvant treatment modalities is still low; therefore, improving neoadjuvant treatment strategies for rectal cancer is the focus of improving the rate of anus and rectum preservation in rectal cancer patients. The effectiveness of immunotherapy in treating malignant tumors is obvious and has changed the traditional anti-tumor treatment paradigm. However, in colorectal cancer, immunotherapy is limited to a specific group with a small proportion of MSI-H/dMMR, and there is no effective immune response for the majority of MSS/pMMR patients. Radiotherapy has been shown to enhance the efficacy of immunotherapy and promote its antitumor effects.

Sintilimab (development code: IBI308) is a recombinant fully human IgG4-type
anti-PD-1 monoclonal antibody developed by Innovent Biologics (Suzhou), Inc. Meanwhile, the available safety data and pharmacokinetic data demonstrate that the safety of Sintilimab in patients is acceptable. Therefore, it is safe and feasible to use Sintilimab as the immunotherapy drug in this study.

The Department of Anorectal Surgery of Changhai Hospital is a national key discipline, a key discipline of Chinese and Western medicine combined with anorectal disease of the State Administration of Traditional Chinese Medicine, a key discipline of anorectal disease of the Chinese People's Liberation Army, and a key discipline of the Second Military Medical University. At present, there are 104 beds in the whole department, and the annual average rectal cancer surgery volume has been ranked among the top in Shanghai in the past three years. The Department has carried out a preliminary study of neoadjuvant radiotherapy combined with immunotherapy, the results of which showed that 40% of patients with MSS-type ultra-low rectal cancer were in complete clinical response. Accordingly, this study intends to investigate the effect of neoadjuvant radiotherapy combined with immunotherapy on the rate of clinically complete patients with MSS-type ultra-low rectal cancer who cannot conserve anus, with the hope that this treatment mode will improve the rate of organ preservation, enhance the overall treatment effect, and improve the quality of life and long-term survival of these patients.

**Purpose**

We intend to investigate the effect of neoadjuvant radiotherapy combined with immunotherapy on the clinical complete remission rate of MSS-type ultra-low rectal cancer without anus preservation, with the clear expectation that this treatment modality will improve the rate of anus and rectum preservation, enhance the overall treatment effect, and improve the quality of life and long-term survival of patients.

**2. Research Process**

This clinical trial is an open label multicenter randomized controlled clinical study,
consisting of 2 treatment regimens: neoadjuvant radiotherapy combined with immunotherapy and neoadjuvant radiotherapy alone. 180 patients will be enrolled in this study, which will be divided into 3 parts: screening, treatment and follow-up.

**Screening**

Screening is to assess your suitability for this study and to carefully document your general health status by your physician. You will undergo a physical examination, provide past and current medical history and specific treatment, provide for collection of routine blood/blood biochemistry/coagulation/cardiac enzymes/tumor markers/thyroid function; routine urine testing; routine stool; imaging (CT thorax and abdomen, pelvic MRI, colonoscopy); and mismatch repair proteins (MSI status, etc.). You will be enrolled in our study after the screening period if you meet all the inclusion criteria, do not meet any of the exclusion criteria, and agree to continue in the study.

However, your doctor will terminate your continued participation in the trial if:

- Requesting to withdraw from the study for various reasons after being enrolled in the study;
- You are unable to complete the study for various reasons after enrollment in the study
- You are a patient with postoperative imaging and pathology confirmed local recurrence or distant metastases, including liver, pelvis, ovaries, peritoneum.

**Treatment**

1. Neoadjuvant radiotherapy + immunotherapy: using long-course radiotherapy mode, irradiate the primary tumor and high-risk area with a tumor dose of 50 Gy (2 Gy/dose \times 25 times); give capecitabine and 2 courses of PD-1 antibody (200 mg/3 weeks) simultaneously during radiotherapy. Two courses of PD-1 antibody (200 mg/3 weeks) and capecitabine alone or Capeox regimen chemotherapy were applied for 2 cycles after the completion of radiotherapy. At the end, pelvic MRI, endoscopy and digital rectal examination were performed to assess the tumor regression grade. Patients with poor tumor regression or even progression were treated surgically as appropriate; patients with good tumor regression and near complete regression (ncCR) were decided after MDT discussion whether to undergo total transanal tumor resection or to enter the Watch & Wait cohort; patients with complete tumor response were entered into the
Watch & Wait cohort.

2. Neoadjuvant radiotherapy: using long-course radiotherapy mode, irradiating tumor dose of 50 Gy (2 Gy/dose × 25 times) to the primary tumor and high-risk area; giving capecitabine monotherapy simultaneously during radiotherapy, followed by capecitabine monotherapy maintenance or Capeox regimen chemotherapy treatment for 2 cycles. At the end of the treatment, pelvic MRI, endoscopy and anal finger examination were performed to assess the tumor regression grade. Patients with poor tumor regression or even progression were treated surgically as appropriate; patients with good tumor regression and near complete response (ncCR) were decided after MDT discussion whether to undergo surgery or to enter the Watch & Wait cohort; patients with clinical complete response (cCR) entered into the Watch & Wait cohort.

Follow-up

For patients entering the Watch & Wait cohort, we will perform digital rectal examination and colonoscopy every 2 months for the first 2 years, and need to combine with endoscopic narrow band imaging (NBI) observation; pelvic MRI every 4-6 months; blood carcinoembryonic antigen every 4 months; chest and abdominal CT every 6 months. Colonoscopy was performed according to the current oncology follow-up guidelines. If cCR is still maintained after 2 years, colonoscopy and digital rectal examination can be performed every 6 months, and after 3 years, it can be changed to once a year.

If local regrowth of the tumor occurs during the Watch & Wait process, we will perform radical surgery and determine the margins of local resection according to the original tumor borders before treatment; patients who undergo local resection are re-admitted to the Watch & Wait follow-up program for meticulous follow-up; patients with the following high-risk factors for local resection are recommended to undergo radical surgery again: ypT stage \( \geq \) T2, hypofractionated adenocarcinoma, indolent cell carcinoma mucinous adenocarcinoma, choroidal carcinoma, nerve infiltration, and positive cut margins. In case of distant metastases during Watch & Wait, if the local lesion continues to remain in complete respond but distant metastases occur, we will prioritize the distant metastases and the primary lesion can continue to Watch & Wait.
If you have completed study treatment or terminated treatment early, survival follow-up begins every 12 weeks after disease progression or termination of treatment, and your physician will continue to collect information on your serious adverse events related to the study drug, subsequent antitumor therapy, and survival follow-up. If you discontinue study treatment for reasons other than disease progression, your physician will also recommend periodic tumor imaging to assess the current status of your disease.

All tests performed throughout the study are to determine your suitability to continue in the study and to ensure your safety. You will need to tell your doctor about any medications and treatments you are using and receiving. Your doctor will ask you throughout the study if you are experiencing any discomfort with your medications and if you are taking them as prescribed, and your doctor will make detailed notes and give you appropriate treatment.

The blood samples and related test reports you provide will be used for this study only and will not be used for other studies. Blood samples tested in our hospital will be tested in accordance with the relevant hospital regulations and the corresponding reports will be issued. Some blood samples will be collected and sent to the central laboratory for testing in accordance with the study regulations. After laboratory testing is completed, the remaining samples will be destroyed after testing is completed and will not be stored further or used for other studies.
3. Research risks and discomfort

During the screening period, you will need to complete a number of tests and examinations. Complications and tissue damage may occur due to individual patient specificity, disease differences, age, and other factors.

Neoadjuvant radiotherapy may lead to the accumulation of toxic side effects of chemotherapy drugs and increase the probability of cytotoxic reactions, bone marrow suppression, peripheral neurotoxicity, cardiotoxicity, gastrointestinal reactions, and allergic reactions; skin ulceration, injury, and poor anal function caused by radiotherapy; the occurrence of disease progression and distant metastasis; and the possible increase in the difficulty of surgery due to neoadjuvant radiotherapy.

Immunotherapy is a relatively new treatment with certain risks associated with immunotherapy. Adverse events with PD1 monoclonal antibody include the following, most of which are mild or moderate and reversible.

- Serious immunotherapy-related serious adverse reactions (low incidence, about 1-2%, PD-1 associated immune myocarditis, pneumonia, hepatitis, colitis, death)
- Common adverse events: malaise, dizziness, fever, myelosuppression, loss of appetite, nausea, vomiting, diarrhea, constipation, gastritis, alopecia, rash, pruritus, limb edema, peripheral neuropathy, elevated blood glucose, hypothyroidism, hyperthyroidism
- Rare adverse events: allergic reactions, abnormal liver function, elevated creatinine levels, proteinuria, hematuria, renal failure, neuromuscular toxicity, drowsiness, disorientation, lower extremity weakness, motor speech disorders, hyponatremia, pneumonia, etc;
- Very rare adverse events: impaired consciousness, convulsions, myocardial infarction, arrhythmias, death.

In addition to the above adverse events, other adverse events may occur. Although Sintilimab has completed phase I/II clinical studies with controlled safety and is available, some unintended adverse reactions may occur. Prior to the start of treatment, we will actively pre-treat anti-allergy, strengthen cardiac monitoring, closely observe vital signs, discontinue treatment as soon as possible if serious adverse reactions occur, and provide active rescue treatment such as cardiopulmonary function protection.
support and anti-inflammatory storm treatment. Medical staff will closely observe and prevent toxic side effects as much as possible during treatment, actively give appropriate therapeutic measures, and strictly control the application indications and termination conditions to reduce accidents. Because study drugs may disrupt embryonic development, sperm or eggs, women who are pregnant or may become pregnant should not use these study drugs and both men and women should use effective contraception. If you do not agree to use effective contraception during the study and for 6 months after the study is completed, you cannot participate in this study. You should tell your doctor immediately if you or your partner are pregnant or may become pregnant. If you are a woman, you will be excluded from the study after your pregnancy is confirmed. The protocol will likely result in a better quality of clinical survival for patients who meet the enrollment criteria, but the possibility of recurrence cannot be ruled out: patients with local recurrence or distant metastases will be withdrawn from the study in the presence of either local recurrence with radical resection; patients with distant metastases will be treated according to guidelines. These two treatment options after recurrence did not significantly increase the associated surgical risks and treatment costs for patients, and had no significant impact on the survival benefit.

4. Possible benefits of participating in the study

The investigator will introduce you to the study drugs and the study schedule, and participation is completely voluntary. Given the results of existing clinical studies, it is possible that your condition may improve through this study and lead to preservation of the rectum and anus. Your condition will be closely monitored by your doctor after you participate in the study. You may not benefit from this treatment due to treatment failure or other potential disease deterioration, but you and other patients will benefit from the new information about the study drug that will be obtained through this study.

5. Research-related costs

Participate in this study will provide you with 4 courses of PD-1 monoclonal antibody for free, and you will be closely monitored by a physician. If the tumor is not in complete clinical remission at the end of the 4 courses, patients will be responsible for
the cost of radical resection or continued use of PD-1 monoclonal antibody. Patients are responsible for the cost of any treatment other than PD-1 monoclonal antibody required during the course of treatment.

Patients with local recurrence or distant metastases will be followed up according to the guidelines, and any treatment options after recurrence will be the responsibility of the patient. All tests, drugs and surgeries in this study are routine clinical examinations and treatments, and will not increase the cost of patients beyond the routine clinical treatment.

6. Subjects' duties

As a study subject, you have the following responsibilities: to provide truthful information about your medical history and current medical condition; to tell the study doctor about any discomfort you have experienced during this study; not to take restricted medications, foods, etc.; to tell the study doctor if you have participated in other studies recently or are currently participating in other studies. In relation to that, you are expected to inform us of any symptoms that occur to you. Please inform us of any increases and changes in taking your regular medications during your participation in the trial.

7. Voluntary participation

You may choose not to participate in the study or request to withdraw from the study at any time by notifying the investigator that your data will not be included in the study results and that any of your medical treatment and rights will not be affected as a result. Your participation in the study may be terminated by the study physician if you require additional treatment, if you fail to comply with the study plan, if a study-related injury occurs, or for any other reason

8. Privacy

If you decide to participate in this study, your participation in the trial and your personal information during the trial will be confidential. Your blood/urine specimen
will be identified by the study number digit and not by your name. Information that identifies you will not be disclosed to members outside the study team unless your permission is obtained. All study members and study sponsors are asked to keep your identity confidential. Your file will be kept in a locked file cabinet and will be accessible only to the researcher. To ensure that the research is conducted in accordance with regulations, members of the government administration or ethics review committee will have access to your personal information at the research unit, as required. No personal information about you will be disclosed when the results of this study are published.

9. Treatment of Subjects' Injuries

In addition to the necessary transportation and nutrition benefits, if you have an adverse event during the study period that is related to this study and the diagnostic tests required by the study protocol, you will be reasonably compensated based on an objective, comprehensive and scientific definition and assessment of the actual situation in accordance with the "Administrative Measures for Conducting Investigator-Initiated Clinical Research in Medical and Health Institutions (Draft for Comments)" issued by the National Health Care Commission on December 30, 2020 and the requirements of the Changhai Ethics Committee.

10. Other treatment options available

Patients with ultra-low rectal cancer may be treated with surgery and a transverse colostomy; you should consult your study doctor for more information.

You may discuss other treatment options with your doctor to decide whether you will participate in this study. If you decide not to participate in this study, your doctor will still suggest other treatment options that are appropriate for you.

Information about this study and the informed consent form have been reviewed by the institutional ethics review committee of this study. If there is any violation of the study protocol during the trial, you may file a complaint directly with the hospital ethics committee.

If volunteering to participate in this study, you will be required to sign an informed consent form certifying that you have been informed about the study and are
volunteering to participate in this study.
If you have questions about the study, or if you experience any discomfort or injury during the study, or if you have questions about the rights of participants in the study, you can contact Dr. Guangyu Yu or the Institutional Ethics Committee at 18801765121 or 021-31162338.
Informed consent form signature page

I have read this informed consent form.

I have been given the opportunity to ask questions and all questions have been answered.

I understand that participation in this study is voluntary.

I may choose not to participate in this study or withdraw at any time with notice to the investigator without discrimination or reprisal, and any of my medical treatment and rights will not be affected as a result.

The investigator may terminate my participation in the study if I require other treatment, or if I fail to comply with the study plan, or if a study-related injury occurs, or for any other reason.

I will receive a signed copy of the "Informed Consent form".

Subject's name: _______________________
Subjects' contact information: _______________________
Date: _______________________

Agent's name: _______________________
Agent's contact information: _______________________
Date: _______________________

I have accurately informed the subject of this document, he/she has accurately read this informed consent form, and I certify that the subject has had the opportunity to ask questions. I certify that he/she has given voluntary consent.

Researcher's name: _______________________
Researcher's contact information: _______________________
Date: _______________________

(Note: If the subject is illiterate, the signature of a witness is required, and if the subject is incapacitated, the signature of a proxy is required.)
Supplemental Material 2

cCR was mainly evaluated based on digital rectal examination, endoscopy and high-resolution MRI findings.

1. Digital rectal examination: local smooth, no mass or only local stiffness in the original tumor site.
2. Endoscopy: the mucosa was pale, complete and smooth, and the proliferation of capillaries was visible; Or visible scar changes; There are also patients whose tumors disappear completely.
3. High resolution magnetic resonance imaging: T2-weighted low signal in the original tumor site, no diffusion limitation in mrTWI, mrTRG was 1.

(If digital rectal examination conflicts with MRI, endorectal ultrasound is recommended)

There is an intermediate state between cCR and definite residual tumor, namely ncCR, which is judged by the following criteria:

1. Locally normal in digital rectal examination, or only touch less than 2cm of uneven or nodules;
2. Mucosa whitening and/or telangiectasia were observed by endoscopy, but mild mucosal unevenness was observed;
3. High resolution magnetic resonance imaging showed low signal in the original tumor site on T2-weighted, and the mrTRG was 1-2.
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<thead>
<tr>
<th>TRG scale</th>
<th>mrTRG</th>
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<tbody>
<tr>
<td>1</td>
<td>No/minimal fibrosis visible (tiny linear scar) and no tumor signal</td>
</tr>
<tr>
<td>2</td>
<td>Dense fibrotic scar (low signal intensity) but no macroscopic tumor signal (indicates no or microscopic tumor)</td>
</tr>
<tr>
<td>3</td>
<td>Fibrosis predominates but obvious measurable areas of tumor signal visible</td>
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<tr>
<td>4</td>
<td>Tumor signal predominates with little/minimal fibrosis</td>
</tr>
<tr>
<td>5</td>
<td>Tumor signal only: no fibrosis, includes progression of tumor</td>
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